# FETAX FOR HUMAN DEVELOPMENTAL HAZARD IDENTIFICATION

## 1.0 INTRODUCTION AND RATIONALE OF FETAX

### 1.1 Scientific Basis for the Use of FETAX

FETAX is essentially an organogenesis test, and organogenesis is highly conserved across amphibians and mammals. The first 96 hours of embryonic development in *Xenopus* parallel many of the major processes of human organogenesis (ASTM, 1991; 1998). Thus, FETAX should be useful in predicting potential human developmental toxicants and teratogens (ASTM, 1991; 1998). Because *Xenopus* embryos are deficient in mixed function oxidase-dependent metabolic activation processes, the addition of an exogenous MAS to the assay allows for an assessment of the need for bioactivation for a substance or complex mixture to induce teratogenic activity. The assay developers have stated that the inclusion of an exogenous MAS in FETAX should increase the accuracy of the test method for determining if substances are likely to be human developmental toxicants (Bantle et al., 1989; ASTM, 1991; 1998).

### 1.2 Intended Uses of FETAX

#### 1.2.1 Intended Regulatory Uses and Rationale

Because FETAX has been concluded by the developers to be easy, rapid, reliable, and inexpensive, the test (with and without metabolic activation) has been proposed as a screening assay for potential human teratogens and developmental toxicants (i.e., for use in hazard identification but not in risk assessment) (ASTM, 1991; 1998). As a screening test, a positive FETAX response would indicate a potential human hazard while a negative FETAX response would not indicate the absence of a hazard. In the role of a screening assay, a negative response would be followed by *in vivo* mammalian testing, while a positive response would require no further testing unless the investigator is concerned about a potential false positive response (i.e., the positive FETAX response occurs at doses not applicable to the *in vivo* situation). However, regardless of the result obtained, an investigator may conclude that confirmatory testing is

merited based on consideration of supplemental information, such as SAR and other chemical and/or testing information.

### 1.2.2 Currently Accepted Teratogenicity/Developmental Toxicity Test Methods

FETAX is not currently accepted by U.S. Federal agencies as a test for identifying teratogenic or developmental toxicants. U.S. Federal and international regulations pertinent to the potential use of FETAX include the following:

- Under the Clean Air Act (CAA), the EPA requires the registration of fuels and fuel additives. As part of the registration process, there are specific toxicity testing requirements. For *in vivo* fertility assessment/teratology testing (40 CFR 79.63), the rat is the preferred species. If other rodent species are used, justification must be provided.

- Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), teratogenicity and reproduction studies require two-generation testing in two mammalian species (e.g., rat, mouse, rabbit, hamster) for pesticides registered for use on food crops (40 CFR 158.202, 40 CFR 158.340).

- Currently accepted EPA test methods for inhalation developmental toxicity studies require the use of at least two mammalian species (e.g., rat, mouse, rabbit, hamster). If other mammalian species are used, justifications/reasoning for their selection shall be provided (40 CFR 798.4350). Similarly, EPA guidelines regarding test methods for reproduction and fertility toxicants make use of the rat, though in some cases and with justification, other mammalian species can be used (40 CFR 798.4700; 40 CFR 798.4900). For this purpose, pregnant females are exposed to the test agent during most of organogenesis. Shortly before delivery, the pregnant females are sacrificed, the uteri removed, and the contents examined for signs of developmental toxicity. The fetal remains are observed for soft tissue and skeletal defects as well as for resorption.

- Toxic Substances Control Act (TSCA) testing requirements for reproduction and fertility effects call for the use of rats, although other mammalian species are acceptable with justification (40 CFR 799.9380). TSCA prenatal developmental toxicity testing requirements suggest the use of the "most relevant" species, with the rat being the preferred rodent species and the rabbit the preferred non-rodent species (40 CFR 799.9370). EPA provisional guidelines for developmental neurotoxicity recommend the use of rats (40 CFR 795.250).

- Under the Federal Hazardous Substances Act (FHSA), the Consumer Products Safety Commission (CPSC) will evaluate all available evidence from animal and human studies in order to determine whether classification based on developmental toxicity is warranted (16 CFR 150.135). No specific testing is required under FHSA.

- Specific guidelines for evaluation of developmental toxicity under the Federal Food, Drug, and Cosmetic Act (FFDCA) may vary depending on the Center but typically require testing of rats and/or rabbits. The Center for Food Safety and Applied Nutrition identifies testing for reproductive and developmental toxicity under "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food," Rev. 1993 (Redbook II). The Center for Drug Evaluation and the Center for Biologics Evaluation and Research reference to International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines, which again indicate the use of rats and/or rabbits (FR 59 (140): 48749).

- Organization for Economic Cooperation and Development (OECD) guidelines do not explicitly restrict developmental toxicity testing to mammals, although the use of FETAX has not been addressed (OECD 414; OECD 415; OECD 416; OECD 421; OECD 422).

A copy of the current EPA guideline for developmental toxicity risk assessment is provided in **Appendix 13**. The four major manifestations of developmental toxicity are death, structural abnormality, altered growth, and functional deficit. Only the first three are traditionally measured in laboratory animals using the conventional developmental toxicity (also called

teratogenicity or Segment II) testing protocol as well as in other study protocols (e.g., multigenerational). As described in this document, the most commonly used protocol for assessing developmental toxicity in laboratory mammals involves the administration of a test substance at three dose levels to pregnant animals (usually mice, rats, or rabbits) during the period of major organogenesis. Treatment is followed by evaluation of maternal responses throughout pregnancy, and then examination of the dam and the uterine contents just prior to term. The high dose is selected to produce some minimal maternal or adult toxicity (i.e., a level that at the least produces marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality). The low dose is generally a no observable effect level for adult and offspring effects. The route of exposure in these studies is usually oral, unless the chemical or physical characteristics of the test substance or pattern of human exposure suggest a more appropriate route of administration. The developmental toxicity endpoints assessed include mortality (e.g., incidence of total, early, and late fetal deaths/litter), malformations (external, visceral, skeletal), variations (external, visceral, skeletal), growth (body weight), clinical signs (type, incidence, duration, and degree), and gross necropsy and histopathology. Many of the endpoints evaluated in FETAX are qualitatively similar to these endpoints. However, no information on functional deficits, which is required in certain regulatory situations (U.S. EPA, 1991), is provided by the current FETAX protocol.

In terms of these regulations and guidelines, a successfully validated FETAX could serve as a screening assay within a tiered scheme to identify potential human teratogens and developmental toxicants (ASTM, 1991; 1998). The use of FETAX as a screening assay in this scheme is described in **Section 1.2.1**.

### 1.2.3 The Use of FETAX to Assess Potential Human Teratogenic Hazards

Based on initial studies, the accuracy of FETAX without metabolic activation for predicting human developmental toxicants was concluded to be greater than 85% (Courchesne, 1985; Sabourin, 1987; ASTM, 1991; 1998; Finch, 1994). Furthermore, it has been proposed that the incorporation of metabolic activation into the assay should increase this accuracy to at least 95%

(ASTM, 1991; 1998). However, analyses conducted by NICEATM of the current FETAX database did not verify these accuracy values (see **Section 6.0**).

### 1.2.4 Intended Range of Chemicals Amenable to Test and Limits According to Physico-Chemical Factors

In the ASTM FETAX Guideline (1991, 1998), FETAX is considered to be applicable to all chemicals individually or in formulations, and to commercial products or mixtures that can be measured accurately at the necessary concentrations in water. With appropriate modifications, FETAX can be used to conduct tests on aqueous effluents; surface and ground waters; leachates; aqueous extracts of water-insoluble materials; and solid-phase samples, such as soils and sediments, particulate matter, sediment, and whole bulk soils and sediments. The preferred solvent is FETAX Solution, which a prepared water-based solution with a standard pH, alkalinity, and hardness suitable for the growth and survival of *Xenopus* embryos (ASTM, 1991; 1998). If a solvent other than FETAX Solution is used, it must be compatible with *Xenopus* embryonic growth and survival. Testing of water-insoluble materials would be limited by the highest concentration that can be achieved using an appropriate organic solvent. The test method is incompatible with materials (or concentrations of materials) that alter the pH, hardness, alkalinity, and conductivity of the FETAX Solution beyond the acceptable ranges indicated in the ASTM FETAX Guidelines (1991, 1998).

## 1.3 Section 1 Conclusions

The scientific basis for FETAX and its intended use(s) as a screening assay for the identification of potential human teratogens are adequately described. Test limits are defined, but only limited information is available on the complete range of materials amenable to test (see **Section 4**).